PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) **International Patent Classification** [5] : C12N 15/00, 5/10, A61K 48/00, A01K 67/027 — A1

(11) **International Publication Number:** WO 94/16065

(43) **International Publication Date:** 21 July 1994 (21.07.94)

(21) **International Application Number:** PCT/US93/12670

(22) **International Filing Date:** 30 December 1993 (30.12.93)

(30) **Priority Data:**
07/999,100 31 December 1992 (31.12.92) US

(71) **Applicant:** EXEMPLAR CORPORATION [US/US]; 1 Innovation Drive, Worcester, MA 01605 (US).

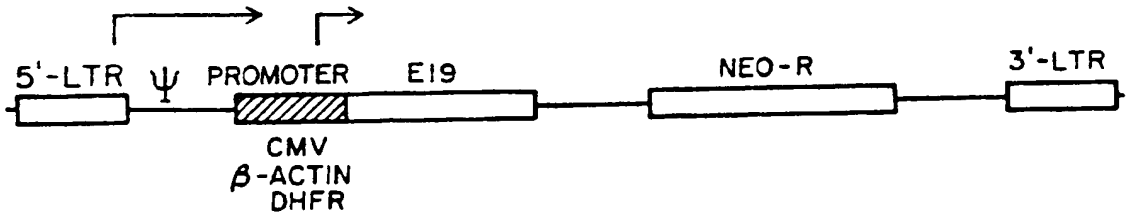
(72) **Inventors:** LEIBOWITZ, Paul, J.; 185 Freeman Street, #446, Brookline, MA 02146 (US). WADSWORTH, Samuel, C.; 12 Ferncroft Road, Shrewsbury, MA 01545 (US). WOON, Chee-Wai; 237 Moreland Street, Worcester, MA 01609 (US).

(74) **Agents:** GATES, Edward, R. et al.; Wolf, Greenfield & Sacks, 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) **Designated States:** AU, CA, FI, JP, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) **Title:** PRODUCING CELLS FOR TRANSPLANTATION TO REDUCE HOST REJECTION AND RESULTING CELLS



(57) **Abstract**

A method for treating cells with E19 protein in order to alter the presentation of MHC class I cell surface antigens on these cells and thereby allow introduction of these cells into a recipient organism while reducing transplant rejection by the recipient organism's immune system is described. The cells may be contacted with E19 protein by the presence in the cells of a vector which carries and expresses the E19 coding sequence or by the presence in the cells of a transgene with expresses the E19 coding sequence. The method may be used for treating a genetic disorder, a wound, a burn, or a disease, or for effecting gene therapy in a recipient organism. Transgenic and chimeric organisms, and cells are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

PRODUCING CELLS FOR TRANSPLANTATION TO REDUCE HOST REJECTION AND RESULTING CELLS

Field of the Invention

This invention relates to a novel method for producing cells in a donor organism for use in transplantation to a recipient organism which reduces transplant rejection and to organisms having such cells.

Background of the Invention

Transplantation of cells, tissues or organs from one organism to another has long been an objective in medicine. It would clearly be desirable if disorders resulting from genetic defects, disease, wounds or burns could be treated with healthy transplants to alleviate the disorder. Such attempts at treatment, however, have been frustrated because the recipient organism's immune system rejects such transplants.

The immune system has evolved to differentiate self from non-self. Cytotoxic T cells respond to antigens that are present on cell surfaces. T cells function by recognizing foreign antigens in combination with determinants present on their host's own cells. These determinants are cell surface proteins coded for by major histocompatibility complex (MHC) genes, especially MHC class I genes. The MHC proteins are present on the surface of all cell types in an animal. Since MHC class I proteins are highly polymorphic, any two animals are likely to have different MHC proteins. T cells use these differences to distinguish self from non-self. Thus, in transplants between allogeneic individuals (i.e., members of

[illegible] species), the transplant is attacked by the recipient's immune system.

Various approaches have been used in attempts to circumvent this transplant rejection response: (i) transplanting into an immunologically deficient host (e.g., athymic animals), (ii) transplanting into an immunologically compromised host (e.g., an irradiated host or a host treated with an immunosuppressive drug such as cyclosporin), or (iii) transplanting into a syngeneic host. General immunosuppression, however, often results in the recipient being susceptible to infections and cancers, while the use of syngeneic organisms is clearly very limited. It has also been reported that cells containing a gene for MHC antigen which is inactivated by homologous recombination may be used as donor cells to an allogeneic recipient. Problems with such an inactivation strategy, however, include: (i) the requirement for inactivation of both alleles of the MHC gene; (ii) an essentially irreversible genetic alteration is required which limits the utility of the method; (iii) such strategies are currently limited to cells that can be readily maintained in culture; and (iv) such strategies cannot be employed efficiently for the production of transgenic animals in species for which embryonic stem cells do not exist.

Recently, researchers pursuing studies relating to viral infection have identified a protein, the E19 protein, from adenoviruses which affects presentation of MHC class I cell surface antigens on cells infected with the adenovirus. Since T-cell receptors simultaneously recognize both the MHC class I cell surface antigens and the surface viral antigens on adenovirus infected cells, the presence of E19 protein impacts on the fate of adenovirus cell infection.

Summary of the Invention

According to the invention, a method of treating a donor organism's cells is provided. These cells are contacted with E19 protein to alter the presentation of MHC class I cell surface antigens on these cells and allow introduction of these cells into a recipient organism while reducing

transplant rejection by the recipient organism's immune system. The cells are provided in a form for introduction into the recipient organism to reduce recipient rejection caused by MHC class I cell surface antigens.

Variations of the method of this invention include subjecting the cells to E19 protein by the presence in the cells of a vector which carries and expresses the E19 coding sequence or by the presence in the cells of a transgene which expresses the E19 coding sequence. The cells that are removed from the donor organism may constitute a tissue or an organ.

The method may also be used for producing a cell in a donor organism for transplantation by introducing DNA into the cell, for example, a vector, that is capable of expressing the E19 protein coding sequence, or by introducing a transgene carrying the E19 coding sequence into a zygote, an early stage embryonic cell, or a stem cell, and allowing this cell to divide and substantially contribute to the development of the donor organism.

The method can be used for gene therapy, or to treat the body for a genetic disorder, a wound, a burn, or a disease in a recipient organism.

In addition, cells for transplantation which are subject to reduced transplant rejection produced by the method of this invention are provided. Transgenic and chimeric nonhuman organisms produced by the method of this invention are also provided.

It is an object of the invention to produce cells which are capable of being transplanted while reducing transplant rejection by the host organism's immune system.

It is another object of the invention to transplant cells, tissues, or organs, into a host organism without

It is another object of the invention to transplant cells, tissues, or organs into a host organism while reducing

the necessity of treating the host to create an immunocompromised host.

It is another object of the invention to introduce into donor cells a source of E19 protein which is capable of altering the presentation of MHC class I cell surface antigens on these cells.

It is another object of the invention to treat a genetic disorder in an organism by transplanting cells into the organism.

It is another object of the invention to treat a wound in an organism by transplanting cells into the organism.

It is another object of the invention to treat a burn in an organism by transplanting cells into the organism.

It is another object of the invention to treat a disease in an organism by implanting cells into the organism.

It is yet a further object of the invention to effect gene therapy in an organism by transplanting cells into the organism.

These and other aspects of the invention, as well as advantages of the invention, will be more apparent from the following detailed description of the invention.

Brief Description of the Drawings

Fig. 1 depicts recombinant retroviral vectors containing the E19 coding sequence fused to the CMV, ß-actin or DHFR promoter.

Fig. 2 depicts three essentially similar fusion constructs with each construct differing substantially only in the promoter region, between the E19 coding sequence and strong general promoters.

Fig. 3 depicts three essentially similar fusion constructs with each construct differing substantially only in the promoter region, between the E19 coding sequence and tissue/organ specific promoters.

Fig. 4 depicts the retroviral vector pWE-Adeno E19 containing the E19 coding sequence fused to the ß-actin promoter.

Fig. 5 depicts a fusion construct between the E19 coding sequence and the rat insulin promoter.

Detailed Description

The prior art reveals the difficulties that have been encountered in transplanting cells, tissues or organs from one organism to another due to rejection of such transplants by the recipient organism's immune system. The present invention describes a method for reducing or eliminating such transplant rejection by altering certain cell surface antigens on the donor cells with adenovirus E19 protein.

According to the invention, a method is provided for treating donor cells to reduce recipient rejection caused by MHC class I cell surface antigens, by contacting the donor cells with E19 protein to alter the presentation of MHC class I cell surface antigens on these cells and allow introduction of these cells into a recipient organism while reducing transplant rejection by the recipient organism's immune system. The cells are provided in a form for introduction into the recipient organism. In some cases, other conventional immunosuppressant means and methods can be used along with the products and methods of this invention.

The term organism is meant to include animals. Animals include mammals, birds, reptiles, amphibians and fish. Preferred animals are mammals and preferred mammals are humans, monkeys, pigs, dogs, cats, sheep, goats, cows, horses and rodents. The most preferred mammal for the recipient organism is a human.

Donor cells for transplantation are obtained from an organism. The term donor cell is meant to include cells, a tissue or an organ. Examples of cells include T cells, B cells, islets of Langerhans, adrenal medulla cells,

retinal epithelial cells, liver cells, bone marrow cells, myoblast cells, hematopoietic cells, spleen cells, cardiac

cells, thymus cells, lung cells, blood cells, glandular cells and stem cells. Stem cells include totipotent cells and pluripotent cells. A totipotent cell means a cell that can differentiate into any cell or tissue type (e.g., an embryonic stem cell or a fertilized egg). A pluripotent cell means a cell that can differentiate into many, but not all tissue types. An example of a pluripotent cell is a hematopoietic stem cell. Examples of tissues include dermal tissue, epidermal tissue, adipose tissue, connective tissue, neuronal tissue, lymphoid tissue, glandular tissue, bone tissue and bone marrow tissue. Examples of organs include kidney, pancreas, liver, heart, lung, gall bladder, skin, spleen, intestine, colon, stomach, eye, inner ear, esophagus, trachea, veins and arteries. The donor cells can be from an organism of the same species as the recipient organism or from a different species than the recipient organism.

The donor cells are contacted with E19 protein. By contacted it is meant that the donor cell is exposed to E19 protein. Preferably, this contact is intracellular. Most preferably, this intracellular contact is the result of intracellular production of E19 protein. The E19 gene encodes a 19 kD glycoprotein from the E3 region of adenoviruses of the subgroups B, C, D and E. (Paabo et al. PNAS USA, 1986, 83: 9665-9669; Wold et al., J. Biol. Chem., 1985, 260: 2424-2431). By E19 protein it is meant the entire 19 kD protein or a portion thereof. By E19 coding sequence it is meant the coding sequence for the entire 19 kD protein or a portion thereof.

The E19 protein has a dominant mode of action. It is believed that E19 protein binds to and prevents MHC class I molecules from exiting the endoplasmic reticulum of the cells. (Paabo et al., Adv. Cancer Res., 1989, 52: 151-163; Burgert et al., PNAS USA, 1987 84: 1356-1360; Cox et al., J. Exp. Med., 1991, 174: 1629-1637). By altering the presentation of MHC class I cell surface antigens on the cell it is meant that MHC class I molecules are at least partially

blocked from becoming cell surface antigens. This alteration in presentation must be sufficient to reduce transplant rejection by a recipient's immune system when the cells are introduced into the recipient organism. Rejection is impairment or destruction of cell structure and/or function to any degree caused by an immune reaction. Preferably, the rejection alleviated by the methods and products of this invention are those cases of rejection where impairment in function and/or destruction of cell structure is such that the desired effects of using the methods and products of this invention are not obtained for a sustained period of time. The term recipient rejection refers to rejection of the transplant by the recipient. The term reduce transplant rejection is meant to include situations where there is no rejection and where there is only partial rejection. The amount of E19 protein that is required for treating the donor cells is that amount which is sufficient to effect such a reduction in transplant rejection. Preferably, the number of E19 molecules is equal to or greater than the number of MHC class I molecules in a given donor cell. Where desired, conventional immunosuppressant therapy can be administered simultaneously with transplants in accordance with this invention. For example, cyclosporin, azathioprine, FK-506 and rapamycin can be administered.

Intracellular contact of the donor cell to E19 protein can be effected in a variety of ways, including introduction into the donor cell of a vector having the E19 coding region, transfection of DNA containing the E19 coding region and production of transgenic animals containing the E19 coding region.

The term vector is meant to include viruses, plasmids, cosmids and YACS. The preferred vector is a virus.

viral vectors.

The genome of conventional recombinant retroviral vectors is comprised of a promoter, a selectable marker and 5' and 3' LTRs (long terminal repeats), as shown in Figure 1. The arrows in the figure show the direction of transcription. The symbol ψ refers to a cis-acting sequence on the 5' end of a retroviral genome distal to the 5' LTR that is required to effect packaging of the RNA genome into retroviral particles. Preferably, the selectable marker on the vector is a neomycin resistance gene (NEO-R) that permits selection with G418. Preferably, the promoter is a strong general or tissue specific internal promoter. Examples of promoters include ß-actin (Lai et al., PNAS USA, 1989, 86: 10006-10010), CMV or DHFR (Scharfmann et al., PNAS USA, 1991, 88: 4626-4630). An example of a retroviral vector containing the CMV promoter is LNL-SLX CMV, an example of a retroviral vector containing the ß-actin promoter is pWE, and an example of a retroviral vector containing the DHFR promoter is LNL-SLX DHFR. The E19 coding sequence is cloned into the genome immediately downstream from the promoter so that the promoter and coding sequence of the E19 gene are operably linked so as to permit expression of the E19 coding sequence. Selective modifications in the sequence of the LTRs to improve the expression of the E19 coding sequence for a variety of cell and tissue types are utilized, as have been described for other cloned genes. (Hilberg et al., PNAS USA, 1987, 84: 5232-5236; Holland et al., PNAS USA, 1987, 84: 8662-8666; Valerio et al., Gene, 1989, 84: 419-427).

Recombinant retroviral vectors capable of transducing and expressing the E19 coding sequence in donor cells are produced by transfecting the recombinant retroviral genome into a suitable (helper virus-free) amphotropic packaging cell line. Examples of such cell lines include PA317 and Psi CRIP (Cornetta et al., Human Gene Therapy, 1991, 2: 5-14; Cone & Mulligan, PNAS USA, 1984, 81: 6349-6353). Transfected virus packaging cell lines produce and package the recombinant retroviral vectors, shedding them into the tissue

culture media. The retroviral vectors are then harvested and recovered from the culture media by centrifugation as described in Compere et al., MCB, 1989, 9: 6-14. The retroviral vectors are resuspended in 10 mM HEPES.

The advantage of using a retroviral vector for introducing the E19 coding sequence into a donor cell is that such vectors, in combination with the choice of an appropriate retroviral packaging cell line, offer wide host range and tissue tropism. Retroviral vectors also permit selective targeting of donor cell and tissue types by appropriate modifications in the LTR.

Recombinant adenoviral vectors also have a cell or tissue specific promoter that is operably linked to the E19 coding sequence so as to permit expression of the E19 coding sequence. An example of the construction of such a vector is essentially as described in Freidman et al., MCB, 1986, 6: 3791-3797; Levrero, et al., Gene, 1991, 101: 195-202. These replication deficient recombinant adenoviral vectors are based on an Ade-5 plasmid. The viral E1 region is deleted from the plasmid pMLP6 (Logan & Shenk, PNAS USA, 1984, 81: 3655-3659) by digesting with the restriction endonucleases BglII and RsaI. The remainder of the plasmid retains the left-most 194 bp of the Ade-5 genome. The entire promoter-E19 fusion gene unit from any of the essentially similar plasmid constructs shown in Figure 2 and Figure 3 is excised with appropriate restriction endonucleases, cut and recloned into the Ade-5 plasmid. The recombinant genomes are generated by mixing the linearized plasmid above with the subgenome fragment of adenovirus DNA representing the 3.85 to 100 map units prepared by digesting the In340 viral genome with ClaI or XbaI. The DNAs are transfected into 293 cells (Graham et al., J. Gen. Virol, 1977, 36: 59-72) as described

segment and each of the cellular promoter-E19 constructs results in the generation of recombinant adenoviral vectors

Recombinant adenoviral vectors infect a wide variety of cells and tissues in susceptible hosts. The host range includes cotton rats, hamsters, dogs, chimpanzees and humans (Hsu et al., J. Infectious Diseases, 1992, 166: 769-775). Such vectors have the distinct advantage of not requiring mitotically active cells for infection.

Viral vectors are used to introduce the E19 coding sequence into donor cells by in vitro or in vivo infection. In in vitro infection, donor cells from target tissues of an organism are obtained, grown in culture and infected with a recombinant viral vector containing the E19 coding sequence. In in vivo infection, a recombinant viral vector is administered to the organism so as to result in general systemic infection or tissue specific or organ specific infection. For example, aerosol administration of recombinant adenoviral vectors results in infection of respiratory epithelial cells (Hsu et al., J. Infectious Diseases, 1992, 166: 769-775; Rosenfeld et al., Science, 1991, 252: 431-434); stereotaxic inoculation of recombinant Herpes simplex viral vectors results in infection in select regions of the brain (Fink et al., Human Gene Therapy, 1992, 3:11-19); infection with retroviral vectors of mitotically active (regenerating) liver or administration into the portal vein results in persistent expression of the infecting genes (Kalenko et al., Human Gene Therapy, 1991, 2: 27-32).

In addition to viral vectors, plasmids can be used to expose donor cells to E19 protein. The term plasmid is meant to describe a DNA molecule that can replicate autonomously or integrate in a host such as a bacterium or yeast. The DNA molecule can contain, in addition, DNA sequences such as those encoding the E19 protein and a promoter controlling its expression in a mammalian cell. The term plasmid also refers to the same DNA molecule above after the DNA molecule is purified away from the host cell such as occurs when using the plasmid DNA for introduction into mammalian cells, when using portions of the DNA molecule for additional DNA cloning

experiments, and when a portion of the DNA molecule is used in a substantially purified form. Such plasmids may contain fusion constructs between the E19 coding sequence and a strong general promoter or between the E19 coding sequence and a tissue or organ specific promoter. Examples of strong general promoters are the mouse H-2K promoter (Schuh et al., Nature, 1990, 346: 757-760, the human ß-actin promoter (Leavitt et al., MCB, 1984, 1961-1969) and the human CMV promoter (Boshart et al., Cell, 1985, 41: 521-530). Figure 2 depicts such fusion constructs. Examples of tissue or organ specific promoters are the liver specific albumin promoter (Heckel et al., Cell, 1991, 62: 447-456), the cardiac specific alpha myosin heavy chain promoter (Subramaniam et al., J. Biol. Chem., 1991, 266: 24613-24620) and lung alveolar epithelium specific promoter SP-C promoter (Wikenheiser et al., Cancer Res., 1992, 52: 5342-5352). Figure 3 depicts such fusion constructs.

DNA from plasmid constructs can be used to introduce the E19 coding sequence into donor cells grown in vitro by DNA-mediated cell transfection methods. The DNA used for transfection is preferably prepared by the alkali-lysis method and preferably purified on cesium chloride equilibrium gradients as described in Sambrook et al., Molecular Cloning: A Laboratory Manual, CSHL, 1989. Preferably, the DNA is introduced into the donor cells by one of the following methods: calcium phosphate precipitation (Ausubel et al., Current Protocols in Molecular Biology, 1987, Wiley-Interscience.), DEAE-dextran method (Ausubel et al., Current Protocols in Molecular Biology, 1987, Wiley-Interscience), electroporation (Ausubel et al: Current Protocols in Molecular Biology, 1987, Wiley-Interscience), or protoplast fusion (Sandri-Goldin et al., MCB, 1981, 1:

in selection medium. Clonal cell lines that have integrated the selectable marker that is present on the transfecting DNA

are picked by ring cloning, expanded in culture and analyzed for the inheritance of the E19 coding sequence by PCR (Innis et al., PCR Protocols: A guide to methods and applications, Academic Press, 1990) and Southern Blot analysis (Southern, J. Mol. Biol., 1975, 98: 503) of genomic DNA prepared from the clonal cell lines. Expression of the transfected E19 coding sequence in the cells is examined by Northern Blot analysis (Sambrook et al., Molecular Cloning: A Laboratory Manual, CSHL, 1989) of total RNA prepared from the cells and by immunoblot analysis with E19-specific antisera (Wold et al., J. Biol. Chem., 1985, 260: 2424-2431).

The DNA from vectors carrying the E19 coding sequence can also be used to directly target specific tissues or organs of a donor organism in vivo. Examples include (i) receptor mediated endocytosis of intravenously administered DNA for delivery to hepatocytes in the liver (Wu et al., J. Biol. Chem., 1991, 266:14338-14342; Wilson et al., J. Biol. Chem., 267:963-967); (ii) liposomes that encapsulate DNA for delivery (Nicolau et al., PNAS USA, 1983, 80: 1068; Kaneda et al., Science, 1989, 243: 375; Mannino & Gould-Fogerite, Biotechniques, 1988, 6: 682; Weinhues et al., DNA, 1987, 6: 81-89); and (iii) direct injection of DNA into muscle or heart, either in solution or as calcium phosphate precipitates (Wolff et al., Science, 1990, 247: 1465-1468; Ascadi et al., The New Biologist, 1991, 3: 71-81; Benvenisty & Reshef, PNAS USA, 1986, 83: 9551-9555).

Intracellular contact of the donor cell to E19 protein can also be achieved by production of transgenic or chimeric organisms containing the E19 coding region. By transgenic organism it is meant an organism that gains new genetic information from the introduction of exogenous DNA into its own, or into an ancestor's germ line. By exogenous DNA it is meant DNA that is not normally found in the genome of the non-transgenic organism. By chimeric organism it is meant an organism in which some of its cells have gained new genetic information from the introduction of exogenous DNA into the

organism, or an ancestor of the organism, preferably at an embryonic stage. By transgene it is meant an exogenous gene that has been added to the germ line of an organism.

Transgenic or chimeric organisms containing the E19 coding sequence are obtained by introducing into a cell a DNA construct containing the E19 coding sequence. The DNA construct has the E19 coding sequence under the control of a promoter. If specific promoters are used, specific populations of cells, tissues or organs of the transgenic or chimeric organism may be targeted. For example, a 2.3 kb mouse albumin promoter has been reported to direct the expression of genes in the liver of transgenic mice (Heckel et al., Cell., 1991, 62: 447-456), a 3.7 kb human SP-C (pulmonary Surfactant-C) promoter will direct the expression of transgenes to the bronchial and alveolar epithelium of adult lungs (Wikenheiser et al., Cancer Res., 1992, 52: 5342-5352), a 5.8 kb alpha myosin heavy chain promoter will direct the expression of transgenes in both the atrial and ventricular cells of the adult heart (Subramaniam et al., J. Biol. Chem., 1991, 266:24613-24620) and a 660 bp rat insulin promoter will direct the expression of transgenes in the ß-islet cells of the pancreas of transgenic mice (Hanahan, Nature, 1985, 315: 115-122). Other tissue specific promoters to produce transgenic animals can also be used, as described in Jaenisch, Science, 1988, 240; 1468-1474; Hanahan, Science, 1989, 240: 1265-1274.

The expression of the adeno-E19 gene may also be placed under the transcriptional control of a strong general promoter that would direct the expression in a wide range of cells and tissues of the donor. Examples are the human B-actin promoter (Leavitt et al., 1984, 4: 1961-1969) and the 1.9 kb mouse H-2K promoter (Schuh et al., Nature, 1990, 346:

under the control of a suitable promoter is introduced into a progenitor cell of the transgenic or chimeric organism by any

process which results in DNA uptake, including microinjection, electroporation, or retroviral infection. Graham and Van der Ebb, 1973, Virology, 52: 456-467; Perucho et al. 1980, Cell, 22: 9-17; Chu et al., 1987, Nucl. Acids Res., 15: 1311-1326; Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, ed. E.J. Robertson (IRL Press, 1987); and Bishop and Smith, 1989 Mol. Biol. Med. 6: 283-298. Introduction of the DNA into a cell includes introduction into a single cell derived from a somatic or germ cell, into an egg, into a sperm, into a zygote, into an embryo, into a post-natal human or nonhuman organism, into cells derived from an embryo or post-natal human or nonhuman organism, or into a cell from a cell culture, e.g., an embryonic stem cell. If embryonic stem cells are used, after introduction of the DNA into such cells, the cells may be transferred to blastocysts to produce lines of transgenic nonhuman organisms. Preferably, the DNA is injected into the pronucleus of a zygote or is introduced into embryonic stem cells.

The cell containing the DNA with the E19 coding sequence is allowed to divide and substantially contribute to the development of the donor organism. The resulting transgenic or chimeric organisms have their MHC class I molecules downregulated as a result of production of E19 protein in the cells containing the E19 coding sequence. Such organisms are a source of cells, tissues and organs for transplantation.

Once the donor cells have been contacted with E19 protein in one of the ways described above, the cells are provided in a form for introduction into a recipient organism. This form can be no change from the form and physical structure of the cells during the contacting step. Such a form requires that the cells be in an appropriate condition for introduction with regard to factors including physiological state, chemical environment and physical condition.

The donor cells are removed after treatment with E19 by standard procedures known to those skilled in the art. For

example, surgical removal of cells, tissues or organs from whole organisms is effected. If cell cultures are the source of donor cells, cells are removed from the cultures. The removed cells are then transplanted into the recipient. The removed cells may be directly transplanted, or they may be stored prior to transplantation. Examples of methods of transplantation include standard surgical grafting procedures, topical application, or injection into the blood stream, a tissue or an organ of the recipient organism.

In preferred embodiments of this invention, a method is provided for treating a genetic disorder, a wound, a burn, a disease, or effecting gene therapy, whereby cells are transplanted into the recipient organism which can substantially alleviate the abnormal condition, the transplanted cells having been treated with E19 protein to alter the presentation of MHC class I cell surface antigens so as to allow the transplantation to the recipient organism while reducing transplant rejection by the recipient organism's immune system. By substantially alleviate it is meant a complete or partial cure or relief of the abnormal condition.

For example, keratinocytes may be introduced to replace skin in the case of burns or to replace skin removed from the recipient organism for use at another site; islets of Langerhans may be introduced for production of insulin for recipient's suffering from diabetes; retinal epithelial cells may be introduced to treat visual disorders such as macular degeneration; immune cells may be used to treat immune deficiency; and myoblasts may be introduced to treat muscle wasting, such as Duchenne's muscular dystrophy. The number of cells introduced will depend upon the particular abnormal condition and the manner in which the cells are introduced.

Generally, the methods of this invention are for treating an organism to achieve a desired result over a sustained time

period. A sustained time period with regard to the desired result to be achieved is any time period which is long enough to be greater than would otherwise have occurred if the treatment of this invention had not been carried out. Such a time period may be short and yet be useful, since immunosuppressant therapy when used in conjunction with the method of this invention, can provide sustained time periods over hours, days, months and years in some cases. Preferably, the reduction in immune rejection is such that the transplanted cells which may be in the form of tissues, organs or parts thereof, will resist transplant rejection over the life of the organism into which the treated cells are transplanted, although a shorter time period of weeks or less, of resistance to severe damage to the transplanted cells by recipient rejection can be useful. For example, where the transplant is used in veterinarian uses for treating animals who in turn are producing useful substances, any prolonging of life and attendant prolonging production of such substances can be important and useful. In humans, of course, longer time periods of at least a month or more are desirable, with periods of at least six months to a year or more being most desirable.

This invention also includes transgenic and chimeric nonhuman organisms which have cells for transplantation which are subject to reduced transplant rejection by a recipient's immune system as a result of the cells producing E19 protein so as to alter the presentation of MHC class I cell surface antigens. This invention further includes a cell from such a transgenic organism, as well as a cell which has DNA which carries and expresses the E19 coding sequence.

EXAMPLES

Example 1: Generation of Allogeneic or Xenogeneic Epidermal Tissues for Transplantation

A. Construction of a recombinant retroviral vector carrying the E19 gene:

A recombinant retroviral vector carrying the Ade-2 E19 gene is prepared as follows. The Ade-2 E19 gene is isolated on a 540 bp BamHI fragment from the plasmid pCMVgp19Kneo by BamHI restriction endonuclease digestion. The BamHI E19 fragment is then subcloned in the BamHI cloning site of the retroviral vector pWE (Choudary et al., Cold Spring Harbor Symp. Quant. Biol., 1986, 51: 1047-1032) by conventional recombinant DNA techniques (Sambrook et al., Molecular Cloning: A Laboratory Manual, CSHL, 1989). In the final construct (PWE-AdenoEI9) shown in Figure 4, the E19 gene is inserted downstream from the strong internal 275 bp chicken ß-actin promoter. The arrow indicates the direction of transcription. The cis-acting ψ sequence is shown. The retroviral genome also contains a neomycin resistance gene (NEO-R) that permits selection with G418, and 5' and 3' LTRs. The recombinant retroviral construct is propagated as a plasmid in E.coli strain HB101 in the presence of ampicillin.

B. Recombinant retrovirus production:

To obtain recombinant retroviruses, 10 ug of the recombinant retroviral DNA is transfected into the amphotropic retroviral packaging cell line PA317 (Miller & Buttimore, MCB, 1986, 6: 2895-2902) by the calcium phosphate precipitation method (Ausubel et al.; Current Protocols in Molecular Biology, 1987, Wiley-Interscience). Transfected

ug/ml G418 (Gibco/BRL, Grand Island, NY). Clonal viral producing cell lines are established in culture. Viral production is carried out by plating the producer lines at 80% confluence in DMEM supplemented with 10% FBS (Gibco/BRL, Grand Island, NY). Media containing shed virus is harvested 24 hours after plating. Viral titers are determined by infecting NIH 3T3 cells in culture in the presence of Polybrene (8 ug/ml) (Sigma, St. Louis, MO). Viral stocks are prepared by centrifugation at 10,000 g at 40°C for 12 hours. Viral pellets are resuspended in 10mM HEPES containing 0.5% dimethyl sulfoxide and stored at -70°C or in liquid nitrogen before use.

C. Keratinocyte cultivation:

Primary epidermal cells or keratinocytes are established in culture from skin biopsies essentially as described (Parenteau et al., J. Cell. Biochem., 1991, 45: 245-251; Green, Scientific American, 1991, 265: 96-102; Flowers et al., PNAS USA, 1990, 87: 2349-2353). Full-thickness skin biopsies are trimmed with surgical scissors to remove dermal tissues, minced and incubated with 0.125% trypsin/0.1% EDTA (Gibco) for 1-2 hours at 37°C. Dissociated cells are harvested every 30 minutes. Cells are washed in Dulbecco's modified Eagle medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) and resuspended in keratinocyte medium (a 7:3 mixture of DMEM with L-glutamine and Ham's F12 (Gibco) medium supplemented with 0.18 M adenine (Boehringer Mannheim, Indianapolis, IN), 10% FBS, insulin (5 ug) (Sigma), transferrin (5 ug/ml) (Gibco), hydrocortisone (0.4 ug/ml) (Gibco), 2 nM 3,3',5-triiodo-L-thyronine (Sigma)), and cholera toxin 0.1 nM (Calbiochem-Behring, San Diego, CA) (Stockschlaeder et al., Human Gene Therapy, 1991, 2: 33-39). For cultivation, 1-2 x 10^6 epidermal cells are seeded in tissue culture flasks (Corning, Corning, NY) on feeder layer of irradiated (20 Gy) 3T3 fibroblasts (Green et al., PNAS USA; 1979, 76: 5665-5668). Cultures are maintained at 37°C,

5% CO_2 in a humidified incubator. Epidermal growth factor (Chiron, Emeryville, CA) was added to the culture at 10 ng/ml and confluent cultures passaged by trypsinization and replating on irradiated murine feeder layers at a cell density of 1 x 10^6 cell/ml.

D. Transduction of primary keratinocytes in culture

Keratinocytes are infected either by co-cultivation with irradiated (20 Gy) PA317 cells producing the recombinant E19 retrovirus or by conventional infection with recombinant retroviral stocks. Cells are infected at 30% confluency with virus (1 x 10^6 colony-forming units (cfu)/ml) in Polybrene (8 ug/ml) at 37°C for 4 hours. The cells are returned to fresh media overnight supplemented with G418 (500 UG/ml) and fed every 2-3 days until confluency.

The transduced keratinocytes are examined for the expression of E19 by immunoblot analysis of total cell lysates using anti-EI9 antisera. The loss of MHC class I molecules on the cell surface resulting from E19 expression is analyzed by immunofluorescence staining with specific MHC monoclonal antibodies (Pharmingen, San Diego, CA; Harlan, Indianapolis, IN) coupled to fluorescent dyes like Rhodamine or Fluorescein (Sigma, St. Louis, MI).

E. Grafting of keratinocytes:

Transduced keratinocytes are prepared for grafting by seeding in 2 cm x 4 2 cm pieces of a biodegradable mesh composed of polyglycolic acid (Dexon; Davis and Geck Inc., Danbury, Conn.) (Hansbrough, et al, Surgery, 1992, 111: 438-446). 4 x 10^5 cells are seeded on each 2 cm square meshes in DMEM supplemented with 2% Ultroser G, a bovine

[illegible] penicillin, streptomycin and amphotericin B (Gibco/BRL). Mesh-keratinocyte cultures are maintained at 37°C until

confluency is reached (i.e., all surfaces are covered by the tissue matrix as assessed by microscopy) before grafting is performed.

F. Application of epidermal graft to recipients

Epidermal grafts are performed on allogeneic and xenogeneic recipients. In animal experimentation, the procedure is performed with approval of the Animal Research Committee and in accordance with the guidelines of the National Institute of Health. In an example of a small animal recipient, the shaved dorsolateral surface of the torso is washed with povidone-iodine and 70% isopropanol. A 2 cm x 2 cm skin section is excised under anesthesia and mesh-keratinocyte graft of equivalent size is placed over the wound. The graft is sutured in place and dressed. To enhance vascularization of the graft, a 2 mm square piece of Gelfoam (Upjohn, Kalimazoo, MI) containing 2 ug of basic fibroblast growth factor may be applied to the connective tissue with the graft (Louis & Verma, PNAS USA, 1988, 85: 3150-3154).

G. Evaluation of the graft

The survival of the allogeneic and xenogeneic skin graft with the E19 modified epidermis is assessed after recovery from surgery as follows:

(a) Examination of the pathology of skin biopsies taken from the grafts and adjacent skin to look for infiltration of lymphocytes and macrophages as an early indication of graft rejection. Grafts are scored daily and considered rejected when there is greater that 50% escharification or shrinkage. (b) Cells from biopsies of engrafted skin may be cultured and identified by the retention of G418 resistance and expression of E19. (c) The potential to reconstitute the stratified epidermis with well defined stratum granulosum and stratum corneum will represent a full commitment of the allograft or xenograft to terminal differentiation.

Example 1 is repeated with the use of conventional cyclosporin immunosuppressant therapy application to the recipient and advantageous results are obtained.

Example 2: Generation of a Transgenic Pancreatic ß-islet Expressing E19 for Transplantation

A. Construction of a rat insulin promoter-E19 fusion gene

The Ade-2 E19 gene is placed downstream from the rat insulin promoter (Hanahan, Nature, 1985, 315: 115-122). The construct is assembled as follows. The plasmid pRIPI-Tag containing the 660 bp rat insulin promoter linked to the SV40 large T-antigen gene is cleaved with the restriction endonuclease HindIII to remove the entire SV40 T-antigen coding region. Plasmid pCMVgp19neo is constructed by inserting the 540 bp BamHI Ade-2 genome containing the E19 gene into the unique BamHI site of the plasmid vector pcDNA1NEO (Invitrogen, San Diego, CA) so that the E19 gene is under the transcriptional control fo the CMV promoter in the plasmid. The Ade-2 E19 gene containing the complete coding sequence is recovered as a 550 bp HindIII-XbaI fragment from the plasmid pCMVgp19Kneo by cleaving with the restriction endonucleases HindIII and XbaI. The XbaI end of the gene is modified by linker ligation to generate a compatible HindIII end. The E19 and rat insulin promoter gene fragments are purified on agarose gels and ligated to produce the rat insulin promoter-E19 fusion gene construct, by conventional recombinant DNA techniques (Sambrook, et al., Molecular Cloning: A Laboratory Manual, CSHL, 1989), as shown in Figure 5. The arrow indicates the direction of transcription.

B. Production of Transgenic Donor

into the pronucleus of a zygote as described in Hogan, et al., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory,

Cold Spring Harbor, NY (1986); Hammer, et al., Nature, 1985, 315: 680-683. Transgenic donors include pigs, mice, rats, rabbits, sheep, monkeys, cats, dogs, goats, cows and horses.

Transgenic animals are identified by performing PCR (Innis et al., PCR Protocols: A guide to methods and applications, Academic Press, 1990) and Southern Blot analysis (Southern, J. Mol. Biol., 1975, 98: 503) of genomic DNA prepared from tail samples. Transgenic animal lines are established. Expression of the E19 transgene in the ß-cells of the pancreas are examined by Northern Blot analysis (Sambrook et al., Molecular Cloning: A laboratory Manual, CSHL, 1989) of total RNA prepared from the islets and by immunoblot analysis with E19-specific antisera (Wold, et al., J. Biol. Chem., 1985, 260: 2424-2431). The ability of E-19 to reduce MHC class I presentation on the cell surface is examined by immunofluorescence analysis of pancreatic tissue sections with specific anti-MHC monoclonal antibodies.

C. Isolation of islet tissues for transplantation

Pancreas are harvested from transgenic animals essentially as described in Nakazato et al., 1992, Surgery, 111: 37-47. The pancreatic ducts are cannulated and the excised organ is immersed in ice cold Hanks' balanced salt solution (HBSS). The organ is then perfused with Type X collagenase (Sigma) in HBSS (1 mg/ml), 10 ml/min at 37°C essentially as described in Alderson et al., Transplantation, 1987, 43: 579-581). The capsule around the glands is removed. The perfusion is terminated when the glands turn mucoid. The tissues are then removed from the ductal system, disaggregated in ice-cold HBSS and filtered through a 500 um stainless steel sieve. The filtrate is concentrated at 800 g for 4 minutes at 4°C. The tissues are stored resuspended in HBSS at 4°C. For transplantation, the tissues are subjected to a final centrifugation at 800 G for 4 minutes and resuspended in a fluid/tissue volume of 2:1 in HBSS.

For small animal donors like a rat, the procedure for the recovery of transplantable islets is described in Lacy & Kostianovsky, Diabetes, 1967, 16: 35-39; Ballinger & Lacy, Surgery, 1972, 72: 175-186. Briefly, the pancreas is recovered by a midline laparotomy in donor animals. The glands are finely chopped with dissecting scissors in HBSS containing 10% calf serum at 4°C to minimize autodigestion. The diced pancreas is then subjected to digestion by collagenase (5 mg/ml). The digestate is collected and islets purified by centrifugation on dialyzed Ficoll (Sigma) step gradient (9.9, 17.1, 18.7 and 20% (W/W) Ficoll solution) at 300,000 g. The islets will settle between the top two concentrations and may be recovered, washed in cold HBSS and used directly for implantation.

The ability of the freshly prepared islets to secrete insulin in response to glucose stimulation is examined (Lacy et al., Diabetes, 1972, 21: 987) by radioimmunoassay (Downing et al., Transplantation, 1980, 29: 79-83).

D. Transplantation of intact transgenic pancreatic islets into recipients

Procedures for the transplantation of pancreatic islets into rodents (Ballinger & Lacy, Surgery, 1972, 175-186), larger animals (Alderson et al., Transplantation, 1987, 43: 579-581) and humans (Tzakis et al., Lancet, 1990, 336: 402-405; Robertson, New England J. Med., 1992, 327: 1861-1868) have been described. In rats, four to six hundred islets are implanted in an anterior thigh muscle pocket. In dogs, islet implantation is performed by direct puncture into the splenic pulp. In humans, islet preparations are resuspended in HBSS containing 10% human albumin for transplantation. 200,000 to 800,000 islets are infused into

small animal models, diabetes mellitus is induced chemically in syngeneic or allogeneic rats, or in the case of

xenografts, a strain of rats by treatment with Streptozotocin (a trademark product of Upjohn, Kalamazoo, MI, containing 2-deoxy-2-[[methylnitrosoamino)-carbonyl]amino]-D-glucopyranose, a compound isolated from Streptomyces achromogenes.). Diabetes is induced within a week after a single injection of 45 mg/kg of body weight of streptozotocin dissolved in 10mM citrate buffer, pH 4.5 into the tail vein. Animals with serum glucose level in excess of 20 mM/liter are considered diabetic. Animals are used 6-8 weeks after treatment. Following recovery from the surgical implantation of donor transgenic islets (400 islets) grafts are examined for viability and immune rejection by conventional pathological methods. Short term graft function is monitored by assessment of blood glucose, insulin and glucagon levels as described in Alderson, et al, Br. J. Surgery, 1984, 71: 756. Long term autograft, allograft and xenograft function are assessed by analyzing mortality rates following withdrawal from insulin therapy.

Example 3: Production of a Transgenic Heart Expressing E19 for Transplantation

A. Construction of a mouse alpha myosin heavy chain promoter-E19 Fusion Gene

The mouse α-myosin heavy chain 5'-flanking region has been cloned (Gulick, et al., 1991, 266: 9180-9185) and characterized (Subramaniam et al., 1991, 266: 24613-24620). In transgenic mice the 5.8 kb 5'-flanking region included the first two exons and part of the third exon was able to direct the expression of transgene in the atrial and ventricular tissues of the adult heart (Subramaniam et al., 1991, 266: 24613-24620). For the purpose of this invention, the Ade-2 E19 gene is placed downstream from the 5.8 kb α-myosin heavy chain promoter. This is accomplished as follows. The 540 bp Ade-2 E19 gene is recovered as a BamnHI-fragment by digesting the plasmid pCMVgp19Kneo with the restriction

endonuclease BamHI. The ends of the fragment are filled in with Klenow to produce blunt ends that are compatible for ligation with the promoter fragment. The α-myosin heavy chain promoter is isolated as a 1.2 kb MaeIII proximal fragment and 4.65 kb BamHI-NdeI distal fragment from the plasmid a-5.5 (Subramaniam et al., 1991, 266: 24613-24620). The ends of the proximal 1.2 KB fragment are filled in with Klenow and ligated to the 540 bp E19 gene into the SmaI site of the plasmid vector pUC18 (N.E. Biolabs, Beverly, MA) so that the E19 gene is operably linked to the short promoter. The fusion construct excised from this plasmid is 1.7 kb NdeI-BamHI. The NdeI site is located close to the 5'-end of the promoter fragment. The remaining 5'-flanking region of the α-myosin heavy chain promoter is added on by ligating the distal 4.65 kb BamHI-NdeI to the fusion construct above at the NdeI site into the BamHI cloning site of pUC18. Finally, the 850 bp BglII-BamHI SV40 polyanadenylation sequence from the plasmid pSV-neo is added to the 3' end of the construct by conventional recombinant DNA techniques (Sambrook et al., Molecular Cloning: A Laboratory Manual, CSHL, 1989).

B. Production of the transgenic donor

The construct is introduced into the germline of donor as described previously for the rat insulin promoter-E19 construct. Transgenic animals are identified and analysed for the expression of E19 in heart tissue as described earlier for the transgenic islets.

C. Assessment of the antigenicity of transgenic heart tissue

The antigenicity of the transgenic donor heart tissue as

biopsy samples of the donor tissues into allogeneic and xenogeneic hosts as described (Davis et al., Transplantation,

Transgenically modified heart tissues (atrial and ventricular) are prepared from neonatal donors along with reference tissue (e.g., skin) samples from the same, as well as from non-transgenic donors implanted subcutaneously behind the ears. Graft survival is assessed by electrocardiography (Davis et al., Transplantation, 1980, 29: 189-192). Skin grafts are assessed by routine pathology.

D. Cardiac grafting in experimental recipients

A transgenic organ is produced that can be transplanted into a recipient to replace a loss of function in the donor. Preferably, the experimental animals are of matching size to accommodate for cardiac demand of the recipient on the grafted organ.

Transplantation is carried out between allogeneic strains of rats or between xenogeneic rats and hamsters. Techniques for heart transplants for rodents are well described in the literature with modifications employed for the choice of vessels and sites for grafting (Marni & Ferrero, Transplantation, 1987, 43: 575-577; Lee et al., Transplantation, 1982, 33: 438-442; Miller et al., Transplantation, 1985, 39: 555-558). These include the renal vessels, the carotid artery and the jugular, the iliac vessels and the abdominal aorta.

Example 3 is repeated with the use of conventional rapamycin immunosuppressant therapy application to the recipient and advantageous results are obtained.

Example 4: Production of Transgenic Animals

A. Animal sources

Animals suitable for transgenic experiments are obtained from standard commercial sources such as Charles River (Wilmington, MA), Taconic (Germantown, NY) and Harlan Sprague Dawley (Indianapolis, IN). Swiss Webster and female mice are used for embryo retrieval and transfer. $B6D2F_1$ males are used for mating and vasectomized Swiss Webster studs are used

to stimulate pseudopregnancy. Vasectomized mice and rats are obtained from the supplier.

B. Preparation of DNA for transfections and microinjections

DNA clones are cleaved with appropriate enzymes and the DNA fragments are electrophoresed on 1% agarose gels in TBE buffer. The DNA bands are visualized by staining with ethidium bromide, excised, and placed in dialysis bags containing 0.3 M sodium acetate, pH 7.0. DNA is electroeluted into the dialysis bags, extracted with phenol-chloroform (1:1), and precipitated by two volumes of ethanol. The DNA is redissolved in 1 ml of low salt buffer (0.2 M NaCl, 20 mM tris, pH 7.4, and 1 mM EDTA) and purified on an Elutip-D column. The column is first primed with 3 ml of high salt buffer (1 M NaCl, 20 mM tris, pH 7.4, and 1 mM EDTA) followed by washing with 5 ml of low salt buffer. The DNA solutions are passed through the column three times to bind DNA to the column matrix. After one wash with 3 ml of low salt buffer, the DNA is eluted with 0.4 ml of high salt buffer and precipitated by two volumes of ethanol. DNA concentrations are measured by absorption at 260 nm in a UV spectrophotomer. For microinjection, the DNA concentration is adjusted to 3 µg/ml in 5 mM tris, pH 7.4 and 0.1 mM EDTA. Additional methods for DNA preparation are found in Hogan et al., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1986).

C. Procedures for producing transgenic animals through the use of embryonic stem cells

A preferred method for producing transgenic animals for this invention is through the use of embryonic stem cells.

been devised to identify and isolate stem cells with the desired insertion event. (For example, Thomas et al., 1987,

Cell 51: 503-512; Capecchi, 1989, Science, 244: 1288-1292; Joyner et al., 1989, Nature 338: 153-156; Thomas et al., 1990, Nature 346: 847-850; Hasty et al., 1991, Mol. Cell. Biol. 11: 4509-4517). Targeted homologous recombination of DNA molecules microinjected into mouse eggs at a very low efficiency has been reported (Brinster et al., 1989, Proc. Natl. Acad. Sci. USA 86: 7087-7091). Detailed procedures for culturing of embryonic stem cells, introducing DNA molecules into embryonic stem cells, and production of transgenic animals from embryonic stem cells are found in Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, ed. EJ Robertson, IRL Press, 1987.

D. Microinjection procedures

The procedures for manipulation of the rodent embryo and for microinjection of DNA into the pronucleus of the zygote are described in detail in Hogan et al., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1986). In addition, microinjection of DNA molecules into the nucleus of a two-cell embryo is also used to produce transgenic animals. Brinster et al. (1985) Proc. Nat'l. Acad. Sci., USA 82:4438-4442.

Similar methods are used to microinject DNA into eggs and embryos of other mammals. Hammer et al. (1985) Nature 315: 680-683; Murray et al. (1989) Reprod. Fert. Devl. 147-155; Pursel et al. (1989) Science 244: 1281-1288; Pursel et al. (1987) Vet. Immunol. Histopath. 17: 303-312; Rexroad et al. (1990) J. Reprod. Fert. 41 suppl. 119-124; Rexroad et al. (1989) Molec. Reprod. Devl. 1: 164-169; Simons et al. (1988) Biotechnology 6: 179-183; Vize et al. (1988) J. Cell Sci. 90: 295-300; Wagner (1989) J. Cell Biochem. 13B suppl. 164.

Microinjection procedures for fish and amphibian eggs are detailed in Houdebine and Chourrout (1991) Experientia 47: 891-897. Procedures for producing transgenic birds are detailed in Shuman (1991) Experientia 47: 897-905.

Other procedures for introduction of DNA into tissues of animals are described in Sanford et al., US Patent # 4,945,050, July 30, 1990.

E. Procedures for producing transgenic rodents by pronuclear injection

The procedures for production of transgenic mice by pronuclear injection are described in detail in: Hogan et al., 1986, Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Procedures for producing transgenic rats are similar to those for mice (Hammer et al., 1990, Cell 63, 1099-1112).

F. Identification of transgenic mice and rats

Tail samples (1-2 cm) are removed from three week old animals. DNA is prepared and analyzed by both Southern blot and PCR to detect transgenic founder (F_0) animals and the progeny (F_1 and F_2).

G. Production of non-rodent transgenic animals

Procedures for the production of non-rodent mammals and other animals have been described by others. Hammer et al. (1985) Nature 315: 680-683; Murray et al. (1989) Reprod. Fert. Devl. 147-155; Pursel et al. (1989) Science 244: 1281-1288; Pursel et al. (1987) Vet. Immunol. Histopath. 17: 303-312; Rexroad et al. (1990) J. Reprod. Fert. 41 suppl. 119-124; Rexroad et al. (1989) Molec. Reprod. Devl. 1: 164-169; Simons et al. (1988) Biotechnology 6: 179-183; Vize et al. (1988) J. Cell Sci. 90: 295-300; Wagner (1989) J. Cell. Biochem. 13B suppl. 164; Houdebine and Chourrout (1991) Experientia 47: 891-897; Shuman (1991) Experientia 47: 897-905; Knight et al. (1988) Proc. Nat'l. Acad. Sci. USA 85:

H. Identification of other transgenic organisms

An organism is identified as a potential transgenic by taking a sample of the organism for DNA extraction and hybridization analysis with a probe complementary to the transgene of interest. Alternatively, DNA extracted from the organism can be subjected to PCR analysis using PCR primers complementary to the transgene of interest.

Equivalents

Those skilled in the art will be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A method of treating donor cells to reduce recipient rejection caused by MHC class I surface antigens, said method comprising:

contacting said cells with E19 protein to alter the presentation of MHC class I cell surface antigens on said cells and allow introduction of said cells into a recipient organism while reducing transplant rejection by said recipient organism's immune system; and

providing said cells in a form for introduction into said recipient organism to reduce recipient rejection caused by MHC class I cell surface antigens.

2. The method of claim 1 wherein contacting said cells with said E19 protein is carried out by intracellular production of said E19 protein.

3. The method of claim 2 wherein said intracellular production of said E19 protein is carried out by the presence in said cells of DNA which carries and expresses the E19 coding sequence.

4. The method of claim 3 wherein said DNA is a vector.

5. The method of claim 2 wherein said cells are from a transgenic nonhuman organism and said intracellular production of said E19 protein is by the presence in said cells of a transgene which expresses the E19 coding sequence.

7. The method of claim 1 further comprising removing [illegible] from said donor

8. The method of claim 7 further comprising:

introducing said last mentioned cells into a recipient organism, and

treating said recipient organism with an immunosuppressant drug.

9. The method of claim 1 wherein said cells are selected from the group consisting of T cells, B cells, islets of Langerhans, adrenal medulla cells, osteoblasts, osteoclasts, epidermal cells, epithelial cells, endothelial cells, neurons, glial cells, ganglion cells, retinal epithelial cells, liver cells, bone marrow cells, myoblast cells, hematopoietic cells, spleen cells, cardiac cells, thymus cells, lung cells, blood cells, glandular cells and stem cells.

10. The method of claim 1 wherein said cells constitute a tissue.

11. The method of claim 10 wherein said tissue is human tissue.

12. The method of claim 10 wherein said tissue is selected from the group consisting of dermal tissue, epidermal tissue, adipose tissue, connective tissue, neuronal tissue, lymphoid tissue, glandular tissue, bone tissue and bone marrow tissue.

13. The method of claim 1 wherein said cells constitute an organ.

14. The method of claim 13 wherein said organ is a human organ.

15. The method of claim 13 wherein said organ is selected from the group consisting of a kidney, pancreas,

liver, heart, lung, gall bladder, skin, spleen, intestine, colon, stomach, eye, inner ear, esophagus, trachea, veins and arteries.

16. The method of claim 1 wherein said recipient organism is a human.

17. The method of claim 7 further comprising introducing said cells after said removing, into said recipient organism.

18. A method of producing a cell in a donor for transplantation which alters the presentation of MHC class I cell surface antigen on said cell so as to reduce transplant rejection, comprising:

introducing into said cell a vector which carries the E19 protein coding sequence, said vector being capable of expressing said E19 protein coding sequence in said donor organism; and

identifying said cell by determining alteration in the presentation of said MHC class I cell surface antigens.

19. A method of producing a cell in a transgenic nonhuman donor organism for transplantation which alters the presentation of MHC class I cell surface antigens on said cell so as to reduce transplant rejection, comprising:

introducing a transgene carrying the E19 coding sequence into a cell selected from the group consisting of a zygote, an early stage embryonic cell and a stem cell, said transgene being capable of expressing said E19 protein coding sequence; and

allowing said cell containing said transgene to divide and substantially contribute to the development of

20. The method of claim 19 further comprising selecting a cell containing said transgene from said donor organism for transplantation into a recipient organism.

21. The method of claim 20 further comprising removing said cell after selecting from said donor organism.

22. The method of claim 21 further comprising introducing said cell after removing into said recipient organism.

23. In a method of transplanting cells from a donor to a recipient organism wherein cells of said donor are selected and introduced into said organism, the improvement comprising:

treating said cells of said donor with E19 protein to alter the presentation of MHC class I cell surface antigens on said cells so that upon transplantation, transplant rejection by said recipient organism's immune system is reduced.

24. The method of claim 23 wherein said cells are human cells.

25. The method of claim 23 wherein said recipient organism is a human.

26. A method of treating a genetic disorder in a recipient organism having an immune system, comprising:

transplanting into said recipient organism cells which can substantially alleviate said genetic disorder,

said transplanted cells having been treated with E19 protein to alter the presentation of MHC class I cell surface antigens so as to allow said transplantation to said recipient organism while reducing transplant rejection by said recipient organism's immune system.

27. A method of treating a wound having damaged cells in a recipient organism having an immune system, comprising:

transplanting into said recipient organism cells which can substantially compensate for said damaged cells of said wound,

said transplanted cells having been treated with E19 protein to alter the presentation of MHC class I cell surface antigens so as to allow said transplantation to said recipient organism while reducing transplant rejection by said recipient organism's immune system.

28. A method of treating a burn having damaged cells in a recipient organism having an immune system comprising:

transplanting into said recipient organism cells which can substantially compensate for said damaged cells of said burn;

said transplanted cells having been treated with E19 protein to alter the presentation of MHC class I cell surface antigens so as to allow said transplantation to said recipient organism while reducing transplant rejection by said recipient organism's immune system.

29. A method of treating a disease in a recipient organism having an immune system, comprising:

transplanting cells into said recipient organism which can substantially alleviate said disease;

said transplanted cells having been treated with E19 protein to alter the presentation of MHC class I cell surface antigens so as to allow said transplantation to said recipient organism while reducing transplant rejection by said recipient organism's immune system.

30. A method of effecting gene therapy in a recipient organism having an immune system, comprising:

transplanting into said recipient organism cells which produce a gene product which can substantially alleviate the effects of an abnormal genetic condition;

[illegible]

antigens so as to allow said transplantation to said recipient organism while reducing transplant rejection by

[illegible]

31. A method for altering presentation of MHC class I surface antigens on a surface of cells which produce MHC class I antigens, so as to reduce rejection of said cells in a transplant of said cells to a recipient organism, said method comprising:

selecting a cell to be used for transplanting into a recipient organism,

introducing an E19 coding sequence into said last mentioned cell, and

permitting expression of said E19 coding sequence in said last mentioned cell.

32. A transgenic nonhuman organism having cells for transplantation which are subject to reduced transplant rejection by a recipient organism's immune system reacting to exposure to MHC class I surface antigens, said cells producing E19 protein so as to alter the presentation of MHC class I cell surface antigens.

33. A chimeric nonhuman organism having cells for transplantation which are subject to reduced transplant rejection by a recipient organism's immune system reacting to exposure to MHC class I surface antigens, said cells producing E19 protein so as to alter the presentation of MHC class I cell surface antigens.

34. A cell from a nonhuman transgenic organism for transplantation which is subject to reduced transplant rejection by a recipient organism's immune system reacting to exposure to MHC class I surface antigens, having a transgene which expresses the E19 protein coding sequence so as to alter the presentation of MHC class I cell surface antigens.

35. A cell for transplantation which is subject to reduced transplant rejection by a recipient organism's immune system reacting to exposure to MHC class I surface antigens,

having DNA which carries and expresses the E19 protein coding sequence so as to alter the presentation of MHC class I cell surface antigens.

36. A method of treating an organism to achieve a desired result over a sustained time period, said method comprising:

selecting at least one cell in accordance with the cell of claim 35, and

introducing said cell into said organism.

37. The method of claim 36 further comprising said organism being treated with an immunosuppressant drug.

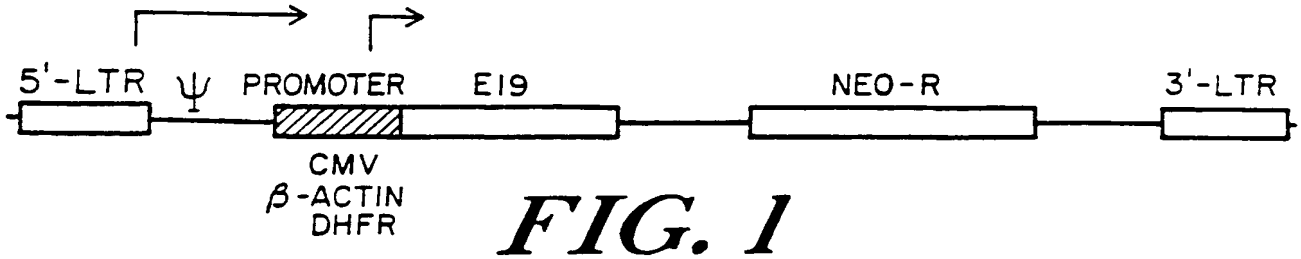

5'-LTR
Ψ
PROMOTER
E19
NEO-R
3'-LTR
CMV
β-ACTIN
DHFR

FIG. 1

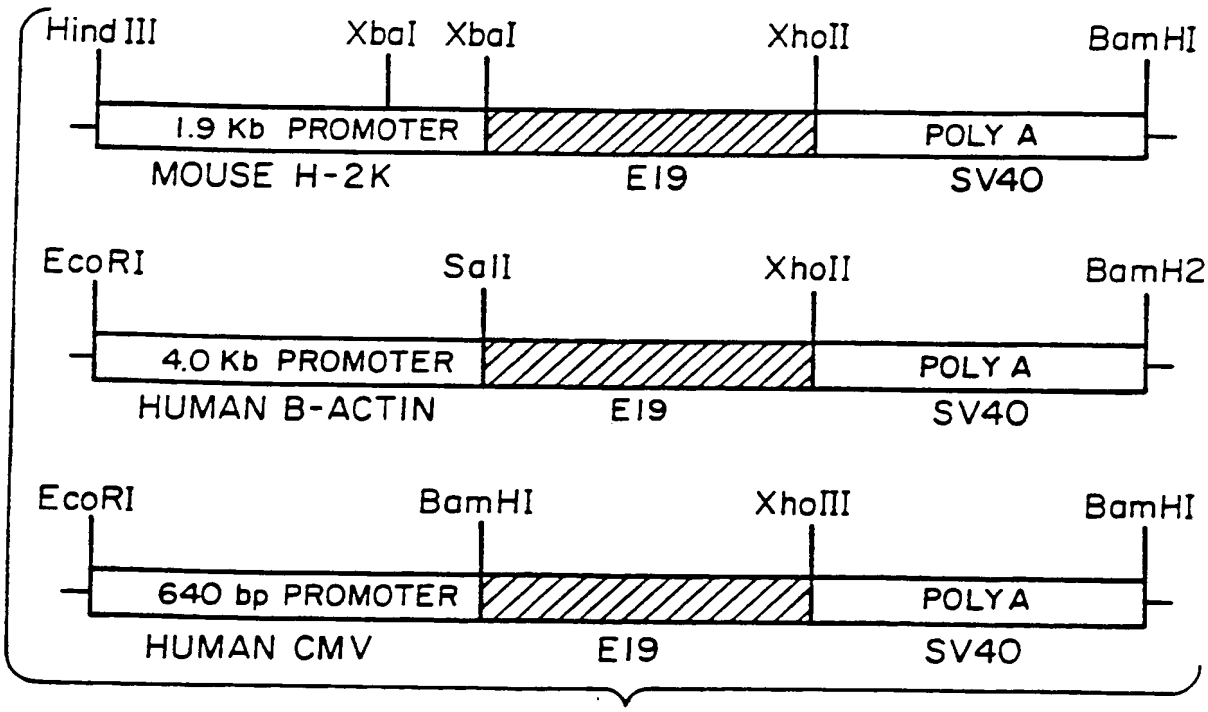

Hind III
XbaI
XbaI
XhoII
BamHI
1.9 Kb PROMOTER
POLY A
MOUSE H-2K
E19
SV40
EcoRI
SalI
XhoII
BamH2
4.0 Kb PROMOTER
POLY A
HUMAN B-ACTIN
E19
SV40
EcoRI
BamHI
XhoIII
BamHI
640 bp PROMOTER
POLY A
HUMAN CMV
E19
SV40

FIG. 2

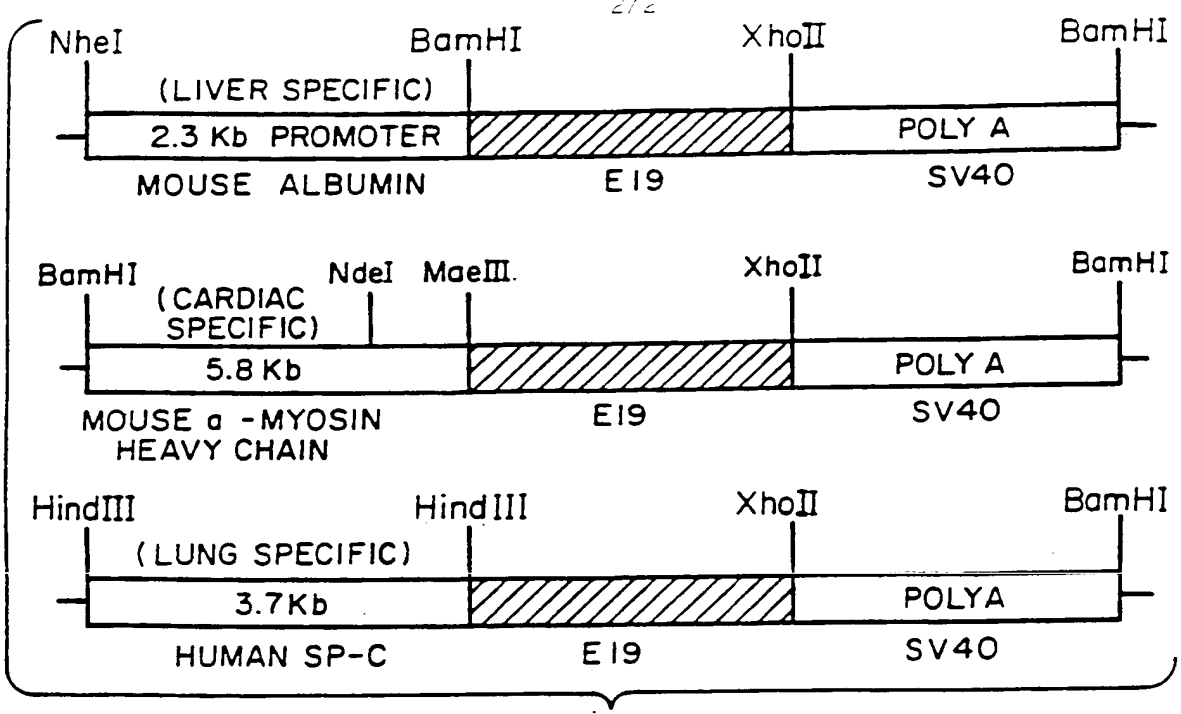


FIG. 3

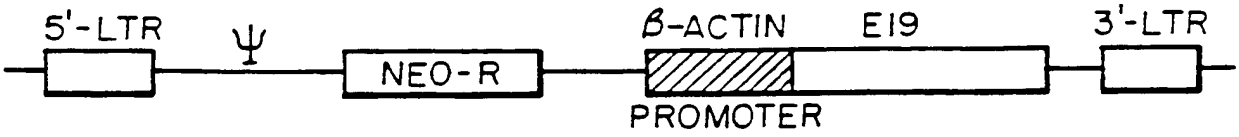


FIG. 4

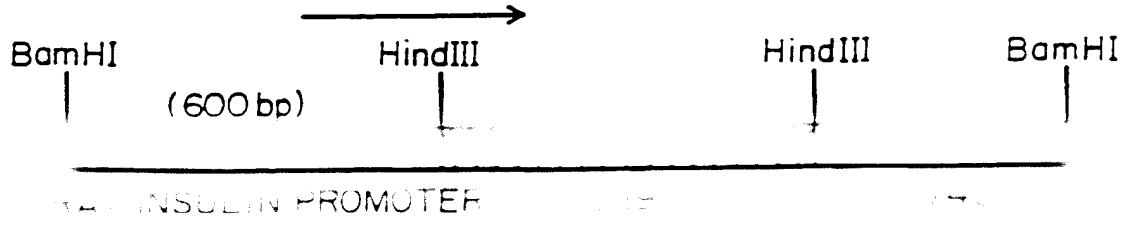


FIG. 5

INTERNATIONAL SEARCH REPORT

Int[illegible]l Application No
PCT/US 93/12670

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/00 C12N5/10 A61K48/00 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A61K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ADVANCES IN CANCER RESEARCH vol. 52 , 1989 , NEW YORK, NY, USA pages 151 - 163 S. PÄÄBO ET AL. 'Adenovirus proteins and MHC expression.' cited in the application see page 154, line 22 - page 159, line 22 see abstract ---	1-4, 6-18, 23-25, 29,31, 33,35,36
Y	WO,A,92 10573 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 25 June 1992 see claims --- -/--	1-4, 6-18, 23-25, 29,31, 33,35,36

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 April 1994	20 -05- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE JOURNAL OF EXPERIMENTAL MEDICINE vol. 174, no. 6 , 1 December 1991 , NEW YORK, NY, USA pages 1629 - 1637 J. COX ET AL. 'Retention of adenovirus E19 glycoprotein in the endoplasmatic reticulum is essential to its ability to block antigen presentation.' cited in the application ---	1-37
A	THE JOURNAL OF IMMUNOLOGY vol. 138, no. 11 , 1 June 1987 , BALTIMORE, MD, USA pages 3960 - 3966 M. ANDERSSON ET AL. 'Reduced allorecognition of adenovirus-2 infected cells.' see abstract see page 3965, right column, line 8 - line 18 -----	1-37

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/US93/12670

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 8, 17, 22-30 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found muluple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all **searchable claims.**

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 93/12670

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9210573	25-06-92	AU-A-	9146991	08-07-92
		CA-A-	2096723	05-06-92
		EP-A-	0560932	22-09-93